Imaging optical fields below metal films and metal-dielectric waveguides by a scanning microscope

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Laser scanning confocal fluorescence microscopy (LSCM) is now an important method for tissue and cell imaging when the samples are located on the surfaces of glass slides. In the past decade, there has been extensive development of nano-optical structures that display unique effects on incident and transmitted light, which will be used with novel configurations for medical and consumer products. For these applications, it is necessary to characterize the light distribution within short distances from the structures for efficient detection and elimination of bulky optical components. These devices will minimize or possibly eliminate the need for free-space light propagation outside of the device itself. We describe the use of the scanning function of a LSCM to obtain 3D images of the light intensities below the surface of nano-optical structures. More specifically, we image the spatial distributions inside the substrate of fluorescence emission coupled to waveguide modes after it leaks through thin metal films or dielectric-coated metal films. The observed spatial distribution were in general agreement with far-field calculations, but the scanning images also revealed light intensities at angles not observed with classical back focal plane imaging. Knowledge of the sub-surface optical intensities will be crucial in the combination of nano-optical structures with rapidly evolving imaging detectors. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.5002071>]

I. INTRODUCTION

Fluorescence labeling and detection are used extensively in cell imaging because they provide the opportunity for selective observation of the molecules of interest,^{1–3} which is not possible using the low contrast provided by wide-field white light transmission images. Because fluorescence occurs almost equally in all directions, fluorescence microscopes use high NA objectives to collect the largest practical fraction of the total emission. However, this results in decreased contrast due to the collection of emission and scattered light from regions of the sample above and below the focal plane of the objective. The introduction of laser-scanning confocal fluorescence microscopy (LSCM) in the mid-1980s provided a revolutionary advance in cell imaging⁴ and provided high-contrast imaging of tissues and cells.^{5,6} The LSCM images are obtained in focal-planes above the glass slide and there was no reason to collect images from below the sample or within the glass slide.

During the past 20 years, there have been advances in the design and fabrication of nanostructures which display unique interactions with light from the UV to near-infrared (NIR) wavelengths. One example is the optical antennas which are able to concentrate propagating optical energy into nanoscale volumes with a sub-wavelength dimension.^{7–10} Another important effect is the ability of photonic and/or plasmonic structures to convert the normally isotropic distribution of fluorescence into directional emission.^{11,12} This effect was first reported for fluorophores within near-field distances of thin Nobel metal

films.^{13,14} The excited state fluorophores created surface plasmons on the metal films which radiated into the glass support at highly confined angles around the Z-axis.^{15–17}

The design and characterization of these fluorescence beaming devices requires a method to measure the angular distribution of light intensity under the nanostructures. These intensity distributions are currently obtained using a prism which allows light propagation into free-space. These measurements are time-consuming even for one-dimensional multi-layer structures with Z-axis symmetry, and becomes impractical with 2D or 3D photonic structures which result in different intensities and polarization at different angles around the Z-axis. An alternative approach is back focal plane (BFP) imaging,^{18–20} which yields the angle-dependent emission intensities around the Z-axis. BFP imaging can be difficult and requires bulky optical components. A disadvantage of BFP images is that they provide no information about the spatial distribution of emission from the XY plane, and thus cannot be used for imaging. Scanning near field optical microscopy (SNOM) can be used to measure such near-field intensity distribution with sub-wavelength resolution.^{21–23} However, the bare tapered or metal-coated fiber tip must be placed into the field to be investigated, which distorts the fields. Additionally, the SNOM fiber tips cannot enter or probe inside the solid supporting materials, such as glass substrates.

We describe the use of a scanning microscope to measure the light intensity distribution in the substrate which supports a multi-layer photonic or plasmonic structure. Such measurements can provide both the angular

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SCHEME 1. Optical configuration for coupled emission detection.

distribution of the emission and spatial resolution in the XY plane.²⁴ Alternative scanning schemes have already been adopted to evaluate the light intensity distribution within micron-scale distances from self-emitting specimens, such as light emitting diodes (LEDs),^{25,26} vertical cavity surface emitting lasers,²⁷ and plasmonic antenna structures.^{28–30} In this paper, we demonstrate measurements of surface plasmon coupled fluorescence emission (SPCE) in three dimensions in the near-field regime with high angular precision. These measurements are motivated by the concept shown in Scheme 1. A fluorescent spot on a structure containing a metal film will create a cone-of-emission at a specific angle from the Z-axis. If the metal film is close to an imaging detector, such as a CMOS camera chip, spatial resolution in the XY plane is retained and the location and number of fluorescent spots can be determined. In contrast, the traditional BFP optics reveals only the angular distribution of the emission and there is no spatial resolution in the XY plane.

II. MATERIALS AND METHODS

The methods used to prepare metal and dielectric films have been described in detail.^{31,32} Briefly, 45 nm thick films of Ag were deposited on clean glass slides by thermal vapor deposition. An additional layer of silica was coated on the bare Ag film by sputtering. The layer thicknesses were determined using a crystal oscillator as described previously.³³ Differences in the thickness of silica on Ag films result in changes of coupled fluorescence emission angles. Two types of samples were investigated. Ag films coated with 0 to 36 nm of silica were used to determine the effect of thin dielectric films on the surface plasmon-coupled emission (SPCE) angles. Waveguide modes are not present with these thin silica films. The second sample was a silver film coated with a 320 nm thick silica layer, which we call a metal-dielectric waveguide (MDW). Using BFP imaging, we recently reported that fluorophores on top of the 320 nm silica layer, but not inside the silica layer, coupled efficiently to modes supported by the MDW and radiated from the bottom of the Ag layers into the glass substrate with a narrow angular distribution.^{34,35} For Ag films coated with thin silica layers (0–36 nm), the fluorophores coupled with the known surface plasmon P (TM)-polarized mode.³⁶ For the sample with a 320 nm layer of silica, the emission coupled with both the P-mode and a S (TE)-polarized waveguide modes. The angular distribution of the coupled emission was measured directly using scanning microscopy as shown in Fig. 1(a).

For these experiments, the structures were drop-coated with fluorescent polystyrene spheres (FPSs) labeled with Nile Red (NR) in water and dried overnight. The diameter of the FPSs is about 2 μm . The FPSs on the structure were excited directly by a linear polarized 532 nm laser beam from the air side and the intensities were imaged using a scanning mirror Olympus FV 300 confocal microscope that was used as a scanning (non-confocal) microscope with

FIG. 1. (a) Schematic configuration of the experimental setup. (b) Emission spectrum of Nile Red molecules in polystyrene spheres recorded through the emission filter. (c) Schematic diagram of fluorescence emission from a randomly oriented dipole, placed on the metal dielectric hybrid structure in the X-Z scanning section. $Z=0$ is located at the Ag-glass substrate interface.

wide-field excitation, Fig. 1(a). Fluorescence was collected by an oil-immersed objective (100 $\times$, N.A. 1.49, Olympus) which was placed on a piezo electric controlled mount to precisely control the distance of the sample to the front lens of the objective. A dielectric coated long-pass edge filter (Cut-On wavelength at 561 nm, Semrock) was placed after the objective to filter the laser beam. The fluorescence was then reflected by the 2D galvanometer before entering the confocal pinhole aperture. Only fluorescence passing through the conjugate area of the pinhole aperture reaches the detector [demonstrated by the black dashed line in Fig. 1(a)].

III. RESULTS AND DISCUSSION

Surface plasmon-coupled emission (SPCE) results from excited state fluorophores within approximately 50 nm from the metal surface. These fluorophores create surface plasmons which transfer energy through the metal film, resulting in a cone-of-emission over a narrow angular range in the metal-coated glass substrate.^{13,14,37} Fluorophores within near-field distances of the MDW can also couple with the structure yielding a cone-of-emission with a narrow angular distribution. These angular emission distributions are typically measured by BFP imaging where the diameter of the emission rings can be used to indirectly determine the angular emission distribution directly below the metal film. For the coupled-emission detection (CED) configuration shown in Scheme 1, it will be necessary to directly measure these sub-surface distributions to obtain spatial resolution in the XY plane as suggested in Scheme 1. These measurements are also needed to test for the presence of evanescent fields or transmitted incident light which is not observed using BFP imaging. We recognized that the Scanning microscope could be used to image the emission intensities under the sample instead of the usual imaging of samples on top of the structure. These considerations suggested that a scanning image in the XY plane should reveal a ring of emission, and an image in the XZ plane should appear as an inverted V-shaped image. When the scanning microscope is used in this way intensities can be measured in the very near-field distances obtained with SNOM (Fresnel region) and the far-field Fraunhofer region.^{38,39}

The use of a scanning microscope to measure the sub-surface fields is demonstrated in Fig. 2 for Ag films with silica layers with increasing thicknesses (0–36 nm) which are too thin to support waveguide modes. Figure 2 presents the X-Z plane intensity distributions measured by scanning microscopy of samples with a silica thickness of 0, 12, 24, and 36 nm. The emission angle is increased with increasing silica thickness, and the measured angles are 43.0°, 44.5°, 48.0°, and 49.5°, respectively. To verify the angular precision of the measurements, we calculated the Attenuated Total Reflectance (ATR) curves³³ of these four samples at 600 nm (Fig. 3). Many reports have demonstrated that the coupled emission angles are equal to decreased reflectivity in the reflectance spectra.^{40,41} SPCE appears to be the reverse of surface plasmon resonance and the calculated surface plasmon resonant (SPR) angles can predict the emission angle of SPCE. The angles measured using a scanning

FIG. 2. Experimental X-Z scanning fluorescence emission pattern from a 2 μm diameter Nile Red FPS on the Ag film coated with different thicknesses of silica: (a) 0, (b) 12, (c) 24, and (d) 36 nm silica. The top edge of each image corresponds to the Ag-Glass interface. The emission angles of these four thicknesses are near 43.0°, 44.5°, 48.0°, and 50.0°, respectively as labeled in the images. The scanning size of these images is 25 μm $\times$ 80 μm .

microscope and simulated ATR spectra are in excellent agreement, within 0.5°, except for one sample with a larger difference. It should be noted that Nile Red-doped in the polystyrene spheres displays a wide emission spectrum with a half-width of 90 nm as demonstrated in Fig. 1(b). The center wavelength is approximately 600 nm, and the major spectrum ranges from 570 nm to 660 nm. Different wavelengths would result in slightly different fluorescence emission angles. Figure 4 presents the angle and wavelength dependent reflectivity of these four samples. The angular change from 570 nm to 660 nm is within 1° to 3°, so exact agreement of the scanning images and SPR angles is not expected.

The images in Fig. 2 reveal that part of the optical energy or emission is centered at 0° along the Z-axis. We did not expect emission close to the Z-axis. For SPCE, we only observed directional emission at large angles.^{13,14} To avoid confusion we note that some incident light is seen along the Z-axis. These observations agree with the simulated reflectance spectra (Figs. 3 and 4) which are for far-field (plane wave) illumination of the sample. In our experiments, the FPSs are within near-field distances from the metal surface. At near-field distances, more optical coupling occurs than with plane-wave illumination where a single well-defined wave vector is incident on the metal surface.^{42,43} To account for both near-field and far-field interactions, we performed numerical simulations of point dipoles using the finite-difference time-domain (FDTD) method.⁴⁴ We simulated the

FIG. 3. Calculated attenuated total reflectance (ATR) curves for metal dielectric structures with silica thickness of (a) 0, (b) 12, (c) 24, and (d) 36 nm for 600 nm wavelength.

intensities in XZ sections of $25\ \mu\text{m} \times 80\ \mu\text{m}$ and which coincide with XZ image planes in Fig. 2. The FPS used in this experiment contained a large number of fluorescent molecules. Illumination with polarized light does not result in a perfectly aligned excited state population, and the emission could be further displayed by homo-fluorescence resonance energy transfer (FRET) between the same types of fluorophores.⁴⁵ To fully evaluate the emission pattern, three

different oriented dipoles were simulated parallel to X-, Y-, and Z-axes as illustrated in Fig. 1(c). Considering the diameter ($2\ \mu\text{m}$) of the FPS used, dipoles at 50 nm and $1\ \mu\text{m}$ from the silver-silica interface were simulated. Coupling between fluorophores and surface plasmons happens within 20–250 nm to the metal surface of the MDWs,^{46–48} and we selected dipoles at 50 nm and $1\ \mu\text{m}$ to represent the coupled and uncoupled situations. Without the loss of generality, a

FIG. 4. Angle and wavelength dependent reflectivity from the SiO_2 layer coated silver film. The thickness of the silver film is 45 nm. The thickness of the SiO_2 layer is (a) 0, (b) 12, (c) 24, and (d) 36 nm. The right color-bar on each panel displays the normalized reflectivity.

FIG. 5. Simulated X-Z sections of emission patterns of 600 nm wavelength dipoles placed on the metal dielectric structure with 12 nm silica. The top edge of each image corresponds to the Ag-Glass interface. Emission patterns of (a) X-, (b) Y-, and (c) Z- oriented dipoles located at 50 nm above the air-silica interface, respectively. Panel (d) shows the total field calculated by adding (a), (b), and (c) in equal proportions. Emission patterns of (e) X-, (f) Y-, and (g) Z- oriented dipoles located at 1 μm above the air-silica interface, respectively. Panel (h) represents the total field calculated by adding (e), (f), and (g) in equal proportion. In order to show the features of the emission patterns clearly, the field intensity on the top edge of each image is saturated and the color-intensity scales are different for each panel. The simulation sizes of these images are 25 $\mu\text{m} \times 80 \mu\text{m}$.

MDW with 12 nm silica was chosen to perform the simulation. Figures 5(a), 5(b), and 5(c) are the emission patterns of X-, Y-, and Z- oriented dipoles located at 50 nm above the Air-silica interface, respectively. Figure 5(d) is the total field distribution added by (a), (b), and (c) in equal proportions. The total field pattern presents as an inverted V-shaped intensity distribution, which matches the prediction from the ATR simulations. For the Y-oriented dipole, the field pattern shows fluorescence emission around 0° , but the intensity is weak compared to the Z-oriented dipole. Hence, in total field pattern this part of emission was not observable. Figures 5(e), 5(f), and 5(g) are the emission patterns of X-, Y-, and Z- oriented dipoles located at 1 μm above the Air-silica interface, respectively. Figure 5(h) is the corresponding total field distribution added by (e), (f), and (g) in equal proportion. The total field pattern demonstrates the emission around 0° for both the X and Y-oriented dipoles. This explains the emission observed around 0° in the scanning microscopy images in Fig. 2. The images in Fig. 5 are intensity normalized but it should be noticed that the emission intensity from a dipole that is 1 μm from the silver-silica interface is much weaker than the coupled emission intensity from a dipole at 50 nm from the silver-silica interface. This is reasonable because excited fluorophores outside the electric field of the surface plasmons cannot couple to surface plasmons and generate only free-space emission that is mostly reflected by the silver film. Considering the size of FPS used, the majority of fluorescence molecules are located out of the coupling region, so the direct transmitted fluorescence is observable. In other words, a combination of Figs. 5(d) and 5(h) closely resembles the measured intensity distribution in Fig. 2.

The emission along the Z-axis was not previously reported for SPCE. For comparison, we collected the BFP images of these samples with identical conditions. The emission angles measured by the BFP method (Fig. 6) agree well with the calculations presented in Fig. 3. However, no direct

transmission fluorescence is observed from the BFP results as compared with Fig. 2. We believe this occurs because the directly transmitted fluorescence is diverging and dissipates during propagation through the BFP optical system. As a result, this component contributes to background noise over the entire observation area. While in the near-field regime of the samples, the propagating length is shorter and the signal is observed. This result illustrates the value of the scanning microscopy images of the sub-surface intensity distributions for the design of integrated devices.

FIG. 6. Back Focal Plane images of fluorescence emission from different MDWs. The thickness of the silver film is 45 nm. The thickness of the SiO_2 layer is (a) 0, (b) 12, (c) 24, and (d) 36 nm. The emission angle is labeled in each picture. The circular dashed line represents the Numerical Aperture (NA) of the objective used in this measurement.

FIG. 7. X-Y scanning sections recorded at different Z values of the MDW with 12 nm thick silica. (a) Intensity pattern recorded on the Ag-Glass interface and intensity patterns recorded at (b) 5, (c) 10, (d) 15, (e) 20, and (f) 25 μm below (negative values) the Ag-Glass interface in the glass substrate. Panels (g), (h), and (i) show intensity patterns recorded at 0, 10, and 25 μm below (negative values) the Glass-Air interface of the glass substrate, respectively. The scanning sizes of these images are $75\text{ }\mu\text{m} \times 75\text{ }\mu\text{m}$.

To obtain additional information about the emission distribution, we recorded images of the XY plane at different Z values, which represent planes at different distances below the metal film. Figure 7 presents the XY scanning sections recorded at different Z values of 12 nm thickness silica MDW. Figure 7(a) is intensity pattern recorded at the Ag-Glass interface. Figures 7(b)–7(f) are intensity patterns recorded on a level of 5, 10, 15, 20, and 25 μm below the silver-glass interface, respectively. The scanning pattern at different levels in the glass substrate reveals a circular

emission ring as expected. The diameter of the rings enlarges with the distance from the silver-glass interface. There is no noticeable intensity difference around the rings, which verifies the nearly unpolarized emission of the differently oriented molecules in the FPSs even when using a linear polarized laser beam. The emission in the center of the rings for small distances below the metal film (–5 and –10 μm) agrees with the measured results in Fig. 2. Control measurements were performed for a FPS on a plain glass slide with no Ag film [Figs. 7(g), 7(h), and 7(i)]. There is no ring of emission and the location of the FPS quickly becomes undetectable. This result demonstrates the need for a suitable plasmonic or photonic structure for CED which maintains spatial resolution in the XY-plane.

In previous reports, we described SPCE from metal films coated with dielectrics with increasing thickness.⁴⁹ In these structures, the directional emission can be P- or S-polarized and appear at different angles. For the MDW with 320 nm thickness silica, there are two resonance modes (TE_1 and TM_1) as demonstrated in Fig. 8(a), which is confirmed in the BFP measurements, images shown in Figs. 8(b) and 8(c). Figure 8(d) presents the measured emission pattern of fluorescence coupled to a MDW with a silica thickness of 320 nm. Comparison of the emission measurement by a scanning microscope with the BFP images reveals two obvious differences: (i) a wide-angle emission near 0° appears in the scanning pattern which was not observed in the BFP measurements. As previously discussed, this part of emission is due to direct transmission of fluorescence. (ii) in the X-Z scanning section recorded by the scanning microscope in Fig. 8(d), only one mode coupled fluorescence emission could be noticed, while in the BFP measurement presented in Figs. 8(b) and 8(c), two modes are clearly demonstrated. The dominant emission angle of 47.0° indicates coupling to the TM mode which is consistent with the BFP images observed through polarizers in Fig. 8(c). To understand our observation of one dominant emission ring and the component along the Z-axis, we simulated the emission patterns of dipoles placed on the MDW with 320 nm silica. Figure 9

FIG. 8. (a) Calculated Attenuated Total Reflectance (ATR) curve of the MDW with 320 nm upper silica layer for 600 nm wavelength. Measured BFP images (b) and (c) and X-Z scanning section (d) of emission from NB on a MDW with 320 nm silica. Panel (c) is the same as that of (b) except (c) is measured with an additional linear polarizer, shown as a double headed arrow in the picture. The scanning size of (d) is $25\text{ }\mu\text{m} \times 80\text{ }\mu\text{m}$.

FIG. 9. Simulated XZ sections of the emission pattern of fluorescence coupled by the MDW with 320 nm silica. The top edge of each image corresponds to the Ag-Glass interface. Emission pattern of (a) X-, (b) Y-, and (c) Z- oriented dipoles located at 50 nm above the silica-air interface. Panel (d) is the total field calculated by adding (a), (b), and (c) in equal proportions. Emission pattern of (e) X-, (f) Y-, and (g) Z-oriented dipoles located at 1 μm above the silica-air interface. Panel (h) is the total field calculated by adding (e), (f), and (g) in equal proportions. In order to show the features of the emission pattern clearly, the field intensity on the top edge of each image is saturated and the color-intensity scales are different for each panel. The simulation size is $25 \mu\text{m} \times 80 \mu\text{m}$.

presents the simulated emission pattern of fluorescence coupled to this MDW. Figures 9(a), 9(b), and 9(c) are the emission pattern of X-, Y-, and Z- oriented dipoles located at 50 nm above the silica-air interface, respectively. It is noticed that the different oriented dipole results in different modes coupled emission in X-Z sections due to the polarization dependence of each mode. Figure 9(d) is the total field distribution obtained by addition of fields from (a), (b), and (c) in equal proportion, only TM_1 mode is appeared, which is in agreement with the scanning microscopy images. In Fig. 9(b), directional emission is observed at 58° , which is due to the TE_1 mode coupled emission. Compared to the TM_1 mode coupled emission simulated in Figs. 9(a) and 9(c), the TE_1 mode intensity is much weaker. In BFP imaging, which is of far-field measurement, the long-distance propagation of the two highly directional emissions is separated from each other; thus, different mode coupled emissions are distinguished. With the scanning microscope, the field is measured at micron distances below MDWs and the different modes still overlap with each other. Because of this overlap and the intensity difference, TE_1 mode is not visible in the total field pattern. Figures 9(e), 9(f), and 9(g) are the emission pattern of X-, Y-, and Z- oriented dipoles located at 1 μm above the silica-air interface, respectively. At this distance, the coupling between the dipole and optical modes in the MDW is no longer present except for the Z-oriented dipoles, and the emission appeared is mainly due to direct transmission of fluorescence. Figure 9(h) is the total field distribution added by (e), (f), and (g) in equal proportion, which explains the wide-angle emission near 0° in Fig. 8(d).

IV. CONCLUSION

We have demonstrated that coupled emission below a multi-layer structure can be recorded directly using a

scanning microscope. Different optical sections of the field distributions can be demonstrated by XZ or XY scanning. The use of a scanning microscope allows fluorescence emission to be recorded in the near-field regime, typically within micron-scale distances from a planar metal-dielectric structure. We examined the MDWs with a thin layer of silica, which possess single mode coupled emission, and MDWs with a thick silica layer that have two modes for coupled emission. The measured results of both structures demonstrated that together with directional coupled emission, there was also a component along the Z-axis. At this time, it is not clear if this component is due to transmitted emission or is a non-propagating evanescent field. In either case, this Z-component may be important when MDWs and other photonics or plasmonic structures are directly coupled to imaging detectors (Scheme 1).

Image formation has long relied on classical far-field optics which makes the imaging systems cumbersome. Development of many consumer products including high-performance cell phones and wearable electronic devices requires further decreases of the size of optical components for integration with the electronics. In our opinion, the combination of modern array detectors with photonic or plasmonic structures provides a pathway to further decrease the size of optical components and to eliminate the need for free-space propagation of light between optical components. This evolution is already in progress as seen by the combination of fluorescent sensors with CMOS chips.⁵⁰ The integration of CMOS detectors with emerging plasmonic and photonic devices will require an understanding of the emission field intensity just below multi-layer and more complex substrates. The use of coupled emission detection (CED), without the need for a focal plane image, can be adequate for many consumer applications and result in greatly simplified devices.

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